

STUDIES ON THE FEMALE REPRODUCTIVE SYSTEM OF THE PRAIRIE DOG, *CYNOMYS LEUCURUS*

II. NORMAL CYCLIC PHENOMENA OF THE OVARIAN FOLLICLES *

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INTRODUCTION

IN PREVIOUS papers the author has outlined the reproductive and seasonal activities and the gross morphology of the female reproductive system of the prairie dog (1929, 1930, 1934). This paper embodies a description of certain normal cyclic phenomena in the ovary.

The investigation was conducted on a series of 192 reproductive tracts from animals collected in Wyoming between March 27 and July 4 in 1925, 1927, and 1928. General descriptions of the material and the technique of its preparation have already been published (1934). Complete serial sections were made of one ovary from each female taken in 1925 and 1927 and of each female taken after parturition in 1928. After histological preparation the ovaries were studied under a Spencer binocular microscope with 15× planoscopic oculars and a 1.8 mm. N.A. 1.25 hom. immersion objective. Measurements were made with an ocular micrometer calibrated against a stage micrometer. Cytological drawings were made with the aid of the equipment listed above and a Spencer camera lucida, yielding a magnification of 2,250 diameters. Photographs were made on "Eastman Wratten M. Plates" in a perpendicular bellows camera box above a Leitz research microscope equipped with achromatic lenses. A Spencer "cold-nose" microscope lamp provided the illumination.

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PRESENT STATUS OF THE PROBLEM

De Graaf (1672), Von Baer (1827), Valentin (1838), Bischoff (1842 to 1854), and Pflüger (1863) made important studies on the structure of the ovary, but were handicapped by inadequate material, primitive technical methods, and a lack of related information. The work of Waldeyer (1870) ushered in a series of modern investigations on the structure and the functions of the ovary.

The development of the ovary must be followed in order to determine the relationships of its many parts. Certain structures which appear during foetal or early postnatal life become vestigial or obliterated before sexual maturity is attained, and others undergo complete change of form, position, or relation during development. During the period from 1900 to 1908 Winiwarter and Sainmont published a series of memoirs on their studies of the ovary of the cat. No other adequate study on the morphogenesis of the ovary of a mammal has been reported. B. M. Allen (1904) and Kingsbury (1913) reported studies which agreed with some results and disagreed with others obtained by Winiwarter and Sainmont. It was thought that a detailed investigation of the structure and development of the ovary in some one mammal might harmonize the findings of these authors in the cat, the pig, and the rabbit and provide a firmer morphological basis for additional investigations.

GENERAL STRUCTURE OF THE MAMMALIAN OVARY

The ovary of the prairie dog, in common with the ovaries of other mammals, presents two major zones, cortex and medulla (Pl. LXXI, Fig. 1). The cortex is a broad, firm peripheral region, interrupted at the hilus and surrounded by a dense tunica ovarii, which is covered by a simple ovarian epithelium. The cortex is made up of Graafian follicles embedded in a stroma of loose connective tissue. The medulla is a central, spongy mass devoid of follicles, but indistinctly delimited from the cortex. It is composed of loose connective tissue, many large blood vessels, nerves, muscle cells, and occasional medullary cords and tubular remains of the rete ovarii. Medullary and rete tissues continue through the hilus into the mesovarium. Embedded in the hilus and mesovarium are epoöphoral vesicles of various sizes and numbers.

Though there is lack of agreement as to the derivation of the

several parts of the mammalian ovary, the following outline, based on the work of B. M. Allen (1904), appears to incorporate the opinions of most recent investigators: (1) The ovarian epithelium gives rise to ova,¹ follicular epithelium, medullary cords, and rete ovarii; (2) The stroma (of mesenchymal origin) differentiates into tunica ovarii, loose connective tissue, thecae folliculi, interstitial cells, and muscle cells; (3) Vascular tissues, nerves, and the epoöphoron invade the ovary from the mesovarium.

GENERAL CYCLIC PHENOMENA

During the life cycle of the prairie dog the curve of physiological activity of the reproductive organs rises rapidly to a crest at puberty and recedes slowly to senility. Seasonal and oestrous cyclic changes are superimposed on the life-cyclic changes. Many of these changes are sufficiently marked to obscure the life-cyclic changes, but the latter overshadow other less conspicuous ones. In ovaries from wild animals of unknown ages it is often impossible to distinguish between life-cyclic, seasonal, and oestrous changes.

The presence of life-cyclic changes is well known in man, and presumably they occur in all mammals, but in none have these changes been followed through a complete life period. Until these changes have been studied over the normal life of some mammal they will continue to be disturbing factors in any morphological investigation of the ovary.

OVARIAN EPITHELIUM

The ovarian epithelium in the adult prairie dog is a simple cuboidal layer covering the tunica ovarii (Pl. LXX, Fig. 1). Its cells have indistinct membranes and relatively large nuclei. They are packed closely together and are high over concave portions, but more widely spaced and low over convex portions of the organ. The nuclei usually appear spheroidal in the high cells and oval in the low ones. On careful examination they are found to be cut into lobules by deep fissures. The chromatin is in the form of an irregular network or in granules, and two or three indistinct karyosomes are present. Mitotic figures are extremely rare. Occasionally cells with

¹ Strictly, "ovum" is the cell after discharge of the second polar body and before the fusion of the male and the female pronuclei. For brevity, the term is here applied to all recognizably potential ova.

large, clear nuclei are seen in the ovarian epithelium. Such cells resemble those described by Hargitt (1930) in the rat as young ova. The possibility that they are young ova is not denied, but they also have the appearance of epithelial cells approaching mitosis.

In extremely rare instances single cells, folds, or columns of cells extend into the tunica ovarii, though retaining their connection with the epithelium (Pl. LXX, Fig. 2). These intrusions usually occur in the area adjacent to the hilus and where the epithelial cells are most crowded. The cells are similar in appearance to the ordinary ovarian epithelial cells, and seem to be forced from the surface rather than to be actively invading the tunica. Occasionally small clumps of epithelial cells are found in the tunica, where its fibers run at an angle to the surface, and numerous cysts of similar cells lie deeper in the ovary (Pl. LXX, Figs. 2-3). The destiny of the cysts is subject to three conjectures, namely, that they become primordial ova, anovular follicles, or both ova and granulosa cells.

In the absence of intermediate stages between the cysts of ovarian epithelial cells and the youngest ova, there is only indirect proof from certain changes within the ovary that the ovarian epithelium in the prairie dog gives rise to ova during sexual maturity. Formation of new ova does not occur during the period covered by this study, and mitosis in the epithelium is not frequent enough to support a cycle of invasion during this period. Furthermore, no ova are recognizable in the epithelium, and intermediate stages between epithelial cells within the ovary and ova are lacking.

OVA

During the period studied all recognizable ova were located in the cortex of the ovary. They are cytologically distinct from the cells of the ovarian epithelium and from the cells of the clumps and cysts beneath it (Pl. LXX, Figs. 1, 3). No satisfactory evidence of a transition from the undifferentiated cells in a cluster to granulosa cells and ova has been discovered. However, the rate of degeneration of ova in the ovary, the small number of ova often encountered, and the absence of invasions of differentiated ova from the epithelium support an inference that they must come from this source, which is in agreement with the belief of Papanicolaou (1924) that ova, once within the stroma of the ovary, pass rapidly through their growth stages to maturity or to degeneration.

Polyovular follicles and nests containing 5 to 15 or more young ova, with few or no follicle cells, are frequently present (Pl. LXXI, Fig. 2). This suggests that one, many, or all of the cells in a cluster may transform into ova and few or none into granulosa cells. However, the preponderance of monovular follicles indicates that typically only one cell of a cluster becomes an ovum. The occasional presence of ova of different sizes in a single follicle may result from a difference in the time of initiation of growth or in the rate of differentiation.

During their period of growth ova pass through the usual sequence of synchronous changes, including the formation of a zona pellucida and its transformation into a zona radiata; increase in volume of the cytosome and in density of the yolk flakes; development of yolk nuclei; growth, later shrinkage, and final disintegration of the nucleus at meiosis; transformation of the chromatin through spireme stages to tetrads, and their separation into dyads; increase in the basichromatin content, subsequent loss of the basichromatin, and final dissolution of the nucleolus.

All recognizable ova in the ovary are enclosed in capsules of granulosa cells. They are approximately 25 micra in diameter when first identified (Pl. LXX, Fig. 3; Pl. LXXI, Fig. 3) and approximately 92 micra when mature (Pl. LXXII, Figs. 3-4). The ovum is surrounded by a distinct membrane. A zone of hyaloplasm encloses the nucleus, and peripherally many yolk granules are found which become larger and more numerous as growth progresses. Small numbers of refractive, acidophilic spherules enclosed in indefinite vacuoles appear in the periphery of ova of diameters of 35 micra and increase in numbers to 100 or more in the larger ova (Pl. LXX, Fig. 3; Pl. LXXII, Fig. 4).

The nucleus of the young ovum is a centrally located sphere 12 to 15 micra in diameter, and is bounded by a distinct nuclear membrane (Pl. LXXI, Fig. 3). Its chromatin is arranged in irregular loops radiating from one or two chromatin nucleoli. The nucleus assumes an eccentric position and increases in size with the ovum until near maturity, when it attains its maximum diameter of 30 micra (Pl. LXXII, Fig. 2), after which it shrinks to approximately 12 micra. Its chromatin becomes detached from the nucleoli, takes on a reticulate appearance, and later is collected into approximately 24 basichromatic spherules. As maturity approaches, the nucleoli

disappear and the spherules of chromatin condense into short, thick masses. Because of their clumping it has been impossible to count these masses accurately. Their number, however, is no less than 20 and no greater than 24. Since this agrees with the count of tetrads on the first maturation spindles, the masses are interpreted as representing early tetrads, and the changes leading up to them as synaptic changes.

Occasionally large ova with first and many with second meiotic division spindles are found. The follicles of all ova observed to be in meiosis, except a single one in first meiotic division, had not reached full size and were in an atretic condition. Meiotic divisions in all immature ova of the period studied are abortive, and the ova are destined to degenerate simultaneously with their follicles. A discussion of the problem of atresia will be presented in a later paper.

A thin hyaline zona pellucida appears around the ovum of approximately 35 micra in diameter, and becomes thicker and more conspicuous around larger ova (Pl. LXX, Fig. 5). As the periovular space becomes progressively wider with the growth of the ovum the zona pellucida is drawn out in the form of an open net, the zona radiata, whose radiating fibers connect the ovum and the granulosa cells (Pl. LXXI, Figs. 4-6).

There are cyclic variations in the rates of origin of ova and in the relative numbers produced, and also of atretic and normal follicles of the various ages. Nests of primordial ova and young ova in follicles are present in ovaries taken throughout the spring and the early summer. The number of young ova is generally greater early in the season, though there are exceptions to this, probably due to individual age differences — the older animals producing fewer young ova and the younger ones producing them over a longer seasonal period. The transformation of epithelial cells into young ova probably occurs before hibernation is broken, since the younger ova are most abundant in early spring and since such transformation has not been observed in animals taken at any time during their active season.

FOLLICLES

Typical follicles may be classed as normal or atretic. Normal follicles are found in various stages of development in the same ovary. Normal or atypical follicles of any size may become atretic

and undergo involution. Only the developmental history of normal follicles is described here.

Normal follicles change greatly in size and in shape during development. In a younger follicle only a single layer of thin cells with flattened nuclei surrounds the ovum (Pl. LXX, Fig. 3). As a result of rapid division the cells become crowded, first into cuboid and then into columnar form (Pl. LXX, Fig. 5; Pl. LXXI, Fig. 4). By continued growth the follicular epithelium retreats from the ovum, leaving a wide periovular space, into which some follicular cells migrate, forming an inner layer (Pl. LXXI, Figs. 4-5). The outer layer retreats from the inner one, leaving an interzonal space. The inner layer in turn retreats from the ovum, leaving another periovular space, across which processes from the cells of the two layers pass to the zona pellucida. Upon further retreat of the granulosa cells, the zona pellucida is drawn out to form a zona radiata (Pl. LXXI, Figs. 4-6).

Cells from both the inner and the outer layers are seemingly crowded into the interzone, where they usually take up irregular positions (Pl. LXXI, Fig. 6). In many follicles these interzonal cells are oriented in rosettes around acidophilic Call-Exner bodies, which are usually replaced later by vesicular spheres (Pl. LXX, Fig. 4; Pl. LXXI, Fig. 6). Spaces appear between the masses of interzonal cells and coalesce into a single antrum, of semilunar form in the immature follicle but of spherical form in the mature one (Pl. LXXII, Figs. 1, 3). In fixed material the contents of the antrum appear granular and contain occasional remnants of the Call-Exner bodies. At the time of the appearance of the antrum the wall of the follicle is approximately six cells in thickness. When the follicle reaches maturity the cumulus oöphorus is spherical and has a wall approximately eight cells in thickness. It separates later from the follicular wall, and is discharged with the ovum at ovulation.

During growth the young follicle acquires a capsule consisting of a theca interna and a theca externa (Pl. LXXII, Fig. 5). The granulosa cells of the outer sphere assume a columnar form and become regularly aligned against the follicular capsule, this condition being maintained throughout the life of the follicle. The cells of the inner sphere are at first pear-shaped, with narrow ends directed away from the ovum, but later they become columnar. They persist in the mature follicle as the corona radiata, attached to the ovum by long

cytoplasmic processes (Pl. LXXII, Fig. 4). The interzonal cells retain an irregular arrangement, or regain it after the destruction of the Call-Exner bodies, and pass from a reticulate to a polygonal form (Pl. LXXII, Fig. 6). Cytoplasmic strands connect all adjacent cells. Processes extend into the antrum from the cells nearest it and to the zona radiata from its neighboring cells.

Follicular growth is accomplished by an increase in the number and the size of the granulosa cells and by the enlargement of the antrum. Mitotic figures are common in the granulosa cells of all immature follicles, but are entirely absent from those of mature ones, and the cells, which are small and thin in the young follicle, become very large as the follicle matures. Their nuclei become correspondingly larger, are usually eccentric in position, and contain from two to six very conspicuous karyosomes and numerous chromatin granules. A paranuclear mass of fibrils is present and occasionally vacuoles are discernible. During the formation of the antrum the cells appear to contain much fluid, suggesting secretory activity. Vascular tissue does not invade the granulosa portion of the follicle.

A follicular capsule, consisting of thin cells with flat nuclei closely applied to the granulosa layer, is present. It is first recognizable when the follicle cells have become cuboid and persists with only a slight increase in thickness until the follicle matures (Pl. LXX, Fig. 5; Pl. LXXII, Fig. 5). The theca interna arises shortly after the follicular cells become cuboid, as a single layer of flattened stromal cells. By frequent mitosis it eventually attains a thickness of approximately six cells (Pl. LXXII, Fig. 5). Some of its cells become polygonal and increase greatly in size, until in the mature follicle they are larger than the granulosa cells. Others remain thin and appear as supporting tissue interwoven among the polygonal cells. The polygonal cells have large, eccentric, oval nuclei, with two or three large karyosomes and an indefinite paranuclear structure. The theca externa develops gradually as the follicles grow and is composed of stromal cells against which the follicle expands (Pl. LXXII, Figs. 3, 5). Mitosis is rare in this layer, and little change occurs in the structure of its cells during development. As the follicle expands it comes in contact with the tunica ovarii from which numerous blood vessels pass into the thecal layer.

The number of available ova is probably the factor which limits

the production of typical follicles. Nests of young ova without follicles appear, but their fate is unknown. Anovular follicles appear (Pl. LXX, Fig. 3), and it is doubtful whether they produce or acquire ova later. Typical follicles follow a definite course in their growth, leading either to atresia or to ovulation. The time and the rate of their formation, the speed with which they develop, and the time at which atresia overtakes some of them determine the relative numbers of follicles of the several sizes in an ovary. Mitosis, hypertrophy, and secretory activity constitute the cytological changes. The lack of knowledge of the age of each animal studied and poorly understood cyclic conditions dependent on age militate against clear-cut interpretations of both classes of changes.

Mature follicles (Pl. LXXII, Fig. 3) are present only at oestrus and have been found only in animals that have a copulation plug in the reproductive canal. Ovulation had occurred in all animals taken after oestrus, and only immature follicles were found in those taken before oestrus. Immature follicles of varying ages were also present in all animals taken before implantation of embryos, and in many animals taken later. Sample counts in all ovaries showed the relative number of very large but immature follicles to be highest before oestrus, to drop to a low number at mid-pregnancy, and to rise again at the period of parturition. After this no change was apparent. The number of large immature follicles, both normal and atretic, was lowest during pregnancy, indicating that fewer follicles pass mid-size at this period. The total absence of mature follicles after oestrus indicates that follicles do not mature after corpora lutea are established.

Middle-sized follicles were numerous in all animals examined, though their number is reduced after the beginning of pregnancy. Atresia is very common in follicles of this size during pregnancy, and during this period the total number of both normal and atretic middle-sized follicles is very high. It is to be inferred that younger follicles reach middle size, at which level they are held by atresia. Younger follicles are numerous throughout the entire active season of the animal and are rarely atretic. In general, the younger follicles predominate early in the season. Their numbers vary, however, in different individuals of the same catch, due possibly to some such factor as variation in age.

Cyclic structural changes also occur in the follicle cells. There

is a wave of mitotic activity in early spring which affects both granulosa and theca cells of all follicles. The mitotic rate remains high during oestrus and early pregnancy, but drops to a low level at mid-pregnancy, where it remains throughout the rest of the active season. During the period from oestrus to implantation there are a marked increase in the size of the theca interna cells and a more intense reaction of the chromatin to stains in all cells of the larger follicles. Secretory activity is inferred from the glandular appearance of these hypertrophied cells.

HISTORY AND DISCUSSION

The germinal nature of the ovarian epithelium in mammals was recognized by Pflüger (1863), who described the ingrowth of cellular cords from it. Valentin (1838) had previously described the origin of ovarian follicles from cords present within the ovary, and Waldeyer (1870) later reported the origin of both granulosa cells and ova from these cords. More recent investigators are generally agreed that a developmental relationship exists between the ovarian epithelium and the ova and granulosa cells.

The nature of the cells composing the ovarian epithelium, however, is still an unsettled question. Waldeyer (1870) believed the epithelium to be made up originally of identical somatic cells, which, after leaving it, might differentiate into several types. Nussbaum (1880) advanced the theory that it was made up of somatic cells, among which were found germ cells derived by early segregation. Lane-Clayton (1905) and Swezy (1929) maintained that it was composed entirely of germ cells. Kingery (1917) held that its cells were all potential germ cells, although of somatic nature. Hargitt (1930) contended that germ cells were not segregated early, but that the ovarian epithelium and, consequently, all its derivatives, such as medullary cords, late foetal follicles, juvenile proliferations, and follicles and ova of the adult were transformed peritoneal cells. He says (1925) that "germ cell origin is a problem of cellular differentiation, and not of early segregation. . . ." His belief follows that of Winiwarter (1900), that a complete degeneration of the earliest recognizable germ cells occurs in the immature ovary and that the idea of a continuity of germ cells is thereby disproved.

Recent investigations favor the theory that the ovarian epithelium of embryos contains potential germ cells. Those who hold that

new ova continue to form during the period of sexual maturity claim that germ cells are also present in the ovarian epithelium of adults. The view that the epithelium is made up of germ cells of an early segregation in addition to somatic cells is also tenable.

Heys (1931) outlines the recent views regarding the origin and the fate of the germ cells of the ovarian epithelium as follows: (1) that the germ cells are not segregated early, but arise entirely by differentiation of somatic cells; (2) that they are segregated early and locate in the gonad, but degenerate before sexual maturity; (3) that they are segregated early, locate in the gonad, and produce definitive germ cells, but that their numbers are augmented periodically by a proliferation, possibly of somatic origin; and (4) that they are segregated early, multiply by mitosis only, and are not augmented or replaced by somatic cells.

Though most recent investigators agree that mammalian ova are derived from the ovarian epithelium, the time and the manner of their derivation and their subsequent history are variously interpreted. Some hold that they invade the ovarian cortex in the young animal only, while others believe that repeated invasions, periodic or continuous, occur throughout life. Many believe the invasion to be by clusters, each cluster differentiating into an ovum and its granulosa cells. Others think that the ovum and its granulosa cells arise by separate invasions. Still others hold that only ova come from the epithelium, granulosa cells arising from the stroma.

According to Waldeyer (1870), the ovum and the granulosa cells are cytogenetically identical; those epithelial cells which stop dividing become ova, whereas those that continue to divide become granulosa cells. Allen (1923) found no group invasions from the epithelium in the adult mouse. He described a displacement of individual cells into the stroma of the ovary after mitosis in the epithelium. Swezy (1929) described a simultaneous origin of ova and follicular cells from the germinal epithelium and regarded them all as sister cells. Follicular cells were distinguished while the ovum was still in the germinal epithelium, and the two types of cell were found to sink into the stroma together. Evans and Swezy (1930) described invasions by one or more seemingly identical epithelial cells without any mitosis immediately preceding the invasion. They held that both ova and granulosa cells came from the invading clumps.

Hargitt (1930b) stated that in the rat new ova are produced in the adult ovary by two methods: (1) Single cells of the germinal epithelium enlarge *in situ* and become surrounded by smaller neighboring cells. The group then moves from its surface position into the tunica albuginea and becomes a young follicle. (2) A number of germinal epithelial cells round up into a mass, and without enlargement of any cells the entire group moves into the albuginea, where one or more cells may grow and transform into ova, whereas others remain small and form follicle cells. League and Hartman (1925) believe that granulosa cells are produced by the epithelium and that they seek out and surround the ova in the stroma of the ovary.

Van Beneden (1880) reported the origin of the ovum and the granulosa cells from a syncytial invasion. Lane-Claypon (1905) held that ova are derived early from the epithelium, serve for a time as interstitial cells, and then change to functional ova. She and McIlroy (1910) described the ovum and its granulosa cells as being derived from the epithelium at different times and held that certain of the cells undergo a partial transformation into ova and then revert to granulosa cells. Jenkinson (1913) did not believe that germ cells in mammals were ever located in the ovarian epithelium but thought that they originated in a subepithelial zone.

The observations on the prairie dog here reported support the view that new ova arise from the ovarian epithelium during sexual maturity. The author considers it unlikely that ova arise from somatic cells, and therefore believes that the ovarian epithelium contains germ cells or indifferent cells of an early segregation. The structural similarity of all cells in the ovarian epithelium in turn supports the idea that it is composed entirely of germ cells or, at least, of germinative tissue capable of producing both functional germ cells and accessory structures. Judgment on its true nature must await the discovery of more reliable cytological criteria for identifying germ cells and improvement in transplantation and tissue culture methods.

The evidence from the study of the prairie dog indicates that groups of similar epithelial cells invade the cortex of the ovary before the annual spring period of activity of the animal and that each group usually differentiates into an ovum and its granulosa cells. None, several, or all such cells may form ova, resulting in an-ovular or polyovular follicles or afollicular nests of ova, respectively.

This interpretation agrees with the reports of Waldeyer (1870), Evans and Swezy (1930), and Hargitt (1930b).

After differentiation the young ova go through a period of rapid growth. The process of yolk formation has been studied in ova of various animals, but homologies are uncertain and interpretations are varied. The presence in the ova of the prairie dog, during their period of growth, of numerous cytoplasmic granules surrounded by clear zones of hyaloplasm suggests that these granules are centers of yolk formation.

Interpretations of the homologies of the egg membranes are not in harmony. Maximow (1931) believed that the zona pellucida was in contact with the protoplasm of the egg and that its radial structure was due to protoplasmic processes traversing it from the granulosa cells to the ovum. He described the outer layer of the zona pellucida as being reticular and attached to the cells of the corona radiata. He believed it to have a dual origin, being derived from both the ovum and the granulosa cells. Arey (1934) states that there is an inner vitelline membrane secreted by the ovum, and that the zona pellucida surrounds the vitelline membrane. Wilson (1925) is of the opinion that the zona pellucida is produced by the ovum, but Corner (1928) thinks that it is produced by follicular cells. Tur (1912), on evidence from polyovular follicles, from follicles without coronae radiata, and from atretic follicles, concluded that the zona pellucida is a product of the ovum. His interpretation is supported by abundant, similar evidence from the ovaries of the prairie dog. In this animal the zona pellucida appears to be a secretion from the ovum laid down outside the vitelline membrane and possibly transformed into a zona radiata by traction of the cells of the corona radiata, which adhere to its outer surface.

Synaptic changes during ovogenesis have been described in many animals. Balfour (1878) observed nuclear changes in the ova of young mammals. Similar observations by later workers were followed by a systematic investigation of ovogenesis in mammals by Winiwarter (1901), who described synaptic changes in the ova of rabbits up to eighteen days of age and took these as a criterion for the identification of germ cells. Winiwarter and Sainmont (1908) reported that in the cat all definitive ova have completed synapsis at four months *post partum* and that no subsequent synaptic changes occur. Pratt and Long (1917) declared that synaptic changes in

the rat ceased at four days *post partum*. Kingery (1917) described indistinct synaptic changes in ovarian epithelial cells in the mouse, but did not consider synapsis necessary in germ-cell history. Cowperthwaite (1925) opposed this view, declaring, as did Winiwarter, that synapsis is a criterion for identification of germ cells, and that its absence in the adult white rat bespoke the absence of ovogenesis. Swezy (1929) interpreted the nuclear changes in young ova of adult rats as modified synapsis. Chromatic activity throughout the period of growth in ova of the prairie dog terminates in a haploid number of chromatic masses. This activity is interpreted as modified synapsis.

Foulis (1876) described the transformation of the early, flat, follicle cells to the cuboid and columnar granulosa cells, their multiplication to form a multilayered body, and a final disintegration of some of the deeper cells with the accompanying formation of a lentiform antrum filled with liquor folliculi. He noted the absence of capillaries in the granulosa layer, and described the vascularization of the theca interna and the hypertrophy of its cells as the follicle approached maturity. The membrana propria surrounding the granulosa layer was described by Balfour (1878), and the follicular capsule of attenuated connective tissue cells associated with the membrana propria was described by Paladino (1888). Call and Exner (1875) described the formation of rosettes of granulosa cells about acidophilic bodies. These Call-Exner bodies may originate through a secretory activity of granulosa cells, in the form either of a liquor folliculi or of a protective secretion analogous to the substance of the zona pellucida about some foreign body as a center. Honoré (1900) attributed the follicular fluid to cell degeneration or to secretion. Lane-Claypon (1905) ascribed all fluid formation to karyolysis of the granulosa cells. Follicular growth in the prairie dog is similar to that in other forms. Foulis and Lane-Claypon have apparently confused karyolysis of the granulosa cells of atretic follicles with the normal production of follicular fluid. The glandular appearance of the granulosa cells and the presence of great numbers of Call-Exner bodies in normal follicles which possess an antrum indicate that cells are not destroyed in the process of producing either follicular fluid or Call-Exner bodies.

Lutein and interstitial tissues have a close genetic relationship to ovarian follicles, though the full extent of these relationships is

not known. Lutein tissue is clearly of dual origin, being formed from granulosa cells and theca interna cells. The origin of interstitial tissue has been variously interpreted. It has been traced to the stroma, to theca interna cells of atretic follicles, to granulosa cells of anovular follicles, and by Lane-Clayton (1905) to functional ova at an intermediate period in their life. In the prairie dog the tissue appears to be of a heterogeneous nature. Shortly after oestrus, cells of the medullary cords, the theca externa, the theca interna, the stroma, the lymphoid tissue, and the epoöphoron hypertrophy and present a marked glandular appearance. Three interpretations may be placed on these structures: (1) Theca-lutein and granulosa-lutein tissues may be physiologically distinct and of independent origin, the first being derived from the stroma and the second from the ovarian epithelium. Likewise, the several types of interstitial cells may be distinct in function and in origin, but synchronous in hypertrophy. (2) All lutein tissues, and likewise all interstitial tissues, may be genetically and functionally identical, differing only in location. (3) They may be passive structures which hypertrophy in response to stimuli from secretions produced by other tissues in the ovary. It is not possible to determine from a morphological study of the adult ovary which of these interpretations is correct.

SUMMARY AND CONCLUSIONS

1. Cytological and histological studies were made on the ovaries of 192 white-tailed prairie dogs taken during their active season, from March 27 to July 4.

2. Oestrous, seasonal, and life-cyclic changes are superimposed in the ovary.

3. During the period covered by this study the ovarian epithelium is a superficial, mitotically quiescent, simple cuboid layer of apparently identical cells. Single cells and small groups of cells projecting from the epithelium into the tunica ovarii, and isolated groups in the tunica ovarii, seldom occur. Groups of identical epithelial cells in the superficial cortex beneath the tunica ovarii are relatively common.

4. All recognizable ova during this period are located in capsules of granulosa cells in the ovarian cortex. The high rate of degeneration of ova and the limited number of young ova suggest that new

ova are formed in the adult. The complete absence of mitosis in ova implies that they originate by a transformation of other cells. The structural similarity of the granulosa cells to the cells of the epithelial groups within the ovary indicates that these groups are the source of follicular cells as well as of the ova. The absence of transitional stages between epithelial cells and ova indicates that transformation does not occur during the period covered in this study.

5. Usually one ovum is found in a capsule of granulosa cells. Polyovular follicles, anovular follicles, and afollicular nests of ova occur rarely. This indicates that typically one cell of an epithelial group transforms into an ovum, whereas the others become granulosa cells. The follicular capsule, the theca interna, and the theca externa originate from the stroma.

6. After invasion, the follicles rapidly complete their life cycle, which terminates in atresia or in ovulation.

7. All ova possess a distinct cell membrane and at an early age produce an elastic zona pellucida, which is later transformed by traction of its granulosa cells into a zona radiata.

8. During growth the ovum increases from a diameter of 25 micra to one of 90, while its nucleus increases from a diameter of 12 micra to one of 30, and becomes eccentrically located in older ova.

9. The nucleus contains a basophilic nucleolus which becomes acidophilic in older ova, and numerous granules, strands, and karyosomes of chromatin, which aggregate into a haploid number of tetrads as maturity approaches.

10. Granulosa cells of growing follicles undergo frequent mitosis, pass from squamous to cuboid or columnar form, and increase greatly in size. In arrangement they pass from a single-layered to a multi-layered sphere, in which the intermediate cells often assume a rosette pattern about Call-Exner bodies. In older follicles they develop a paranuclear structure and become secretory. Interstitial spaces coalesce to form an antrum about 900 micra in diameter when mature. The cumulus oöphorus is discharged into the antrum as a sphere. In older follicles the nuclei of the granulosa cells develop many large karyosomes.

11. The theca interna increases by mitosis in growing follicles and passes from a body of a single layer in thickness to one of 5 or 6 layers. Its cells change from a squamous to a polygonal form and in older follicles exceed the granulosa cells in size.

12. As the follicle enlarges, the theca externa increases in thickness, largely by the addition of stromal cells, to a small extent by cell division.

13. The theca interna becomes progressively more vascular in growing follicles. Blood vessels do not enter the granulosa tissue.

14. Young follicles are most numerous early in the season. Older normal follicles are most numerous before implantation and least so at mid-pregnancy. Mature follicles are present only at oestrus. The rate of follicular growth is at its peak early in the season.

15. Atresia is not prevalent before oestrus, but is common during pregnancy.

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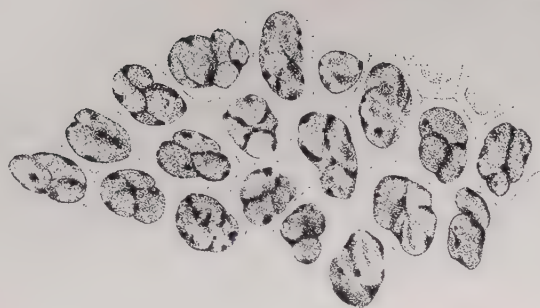
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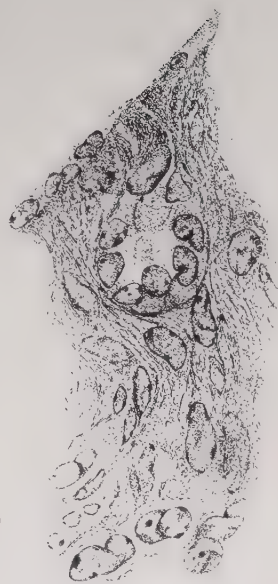
EXPLANATION OF PLATE LXX

- FIG. 1. Ovarian epithelium, superficial view, showing the indistinct cell boundaries, the large, lobulated nuclei, and the uniform structure of the cells
- FIG. 2. Section of the ovarian epithelium and the tunica ovarii, showing the cuboid structure of the epithelium and a cluster of epithelial cells invading the tunica. Another group of epithelial cells with lobulated nuclei is shown deeper in the tunica
- FIG. 3. A typical young ovum in its follicle of flattened granulosa cells. The cell membrane, perinuclear zone of hyaloplasm, and strands of chromatin centering on the nucleolus are shown. Beside the follicle is an anovular cyst of epithelial cells, with lobulated nuclei
- FIG. 4. A cluster of granulosa cells surrounding a Call-Exner body
- FIG. 5. Portion of an early ovum and follicle, showing the early zona pellucida, the follicular capsule, and the theca interna

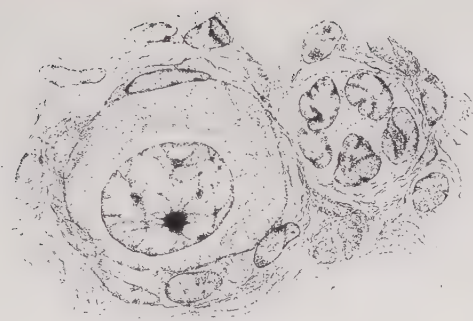
PLATE LXX



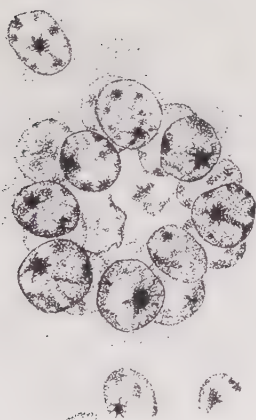
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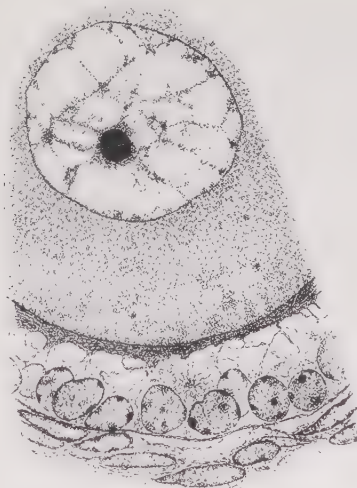
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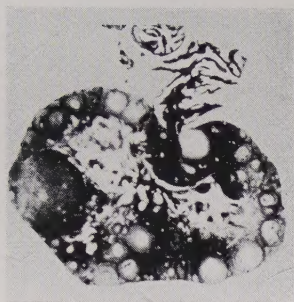


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EXPLANATION OF PLATE LXXI

- FIG. 1. Sagittal section of ovary, showing cortex bearing numerous follicles and a corpus luteum, vascular medulla, hilus, and mesovarium
- FIG. 2. A nest of young ova in the cortex, with no follicle cells near them
- FIG. 3. A young ovum surrounded by flattened granulosa cells. Its nucleus is large, and the chromatin is in the form of heavy strands centering on the nucleolus. Numerous yolk granules and occasional spherules enclosed in vacuoles are shown in the cytosome
- FIGS. 4-5. Ova in follicles whose granulosa cells have formed inner and outer spheres and are transforming the zonae pellucidae into zonae radiatae
- FIG. 6. An older ovum and follicle. The theca externa and the membrana propria, and the inner and outer spheres of granulosa cells and the interzonal cells clustered about Call-Exner bodies are shown. Numerous mitotic figures are seen in the granulosa cells

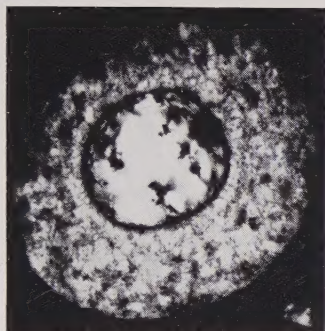
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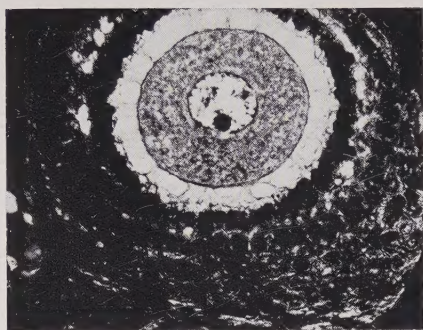
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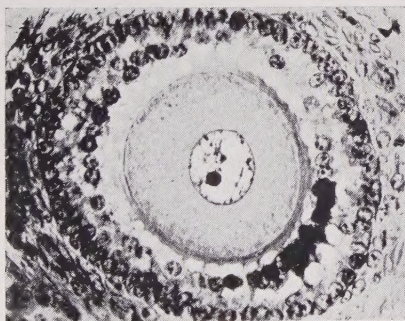
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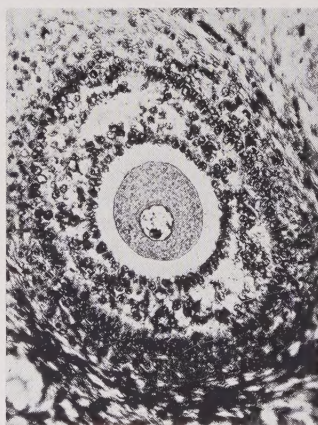
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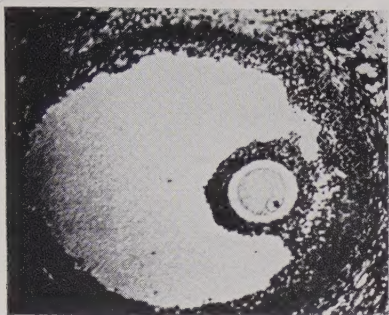


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EXPLANATION OF PLATE LXXII

- FIG. 1. A large, immature follicle possessing a lenticular antrum, into which the cumulus oöphorus, with its ovum, is being discharged
- FIG. 2. Details of a large, immature ovum. The nucleus is shown at its maximum size and with its chromatin material in the form of basicchromatic spherules
- FIG. 3. A mature follicle containing a spherical antrum, in which the cumulus oöphorus is free-floating
- FIG. 4. Ovum and corona radiata of a mature follicle. Paranuclear structures are apparent in the granulosa cells and numerous yolk granules and yolk nuclei in vacuoles are seen in the ovum
- FIG. 5. Details of the theca externa, the theca interna, the membrana propria, and the peripheral granulosa cells of a follicle nearing maturity
- FIG. 6. Details of the interzonal granulosa cells of a follicle nearing maturity

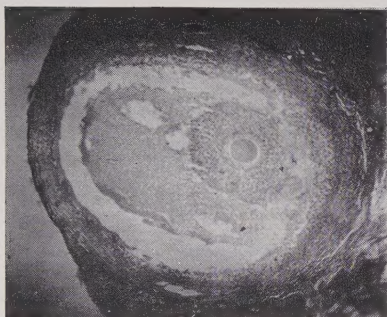
PLATE LXXII



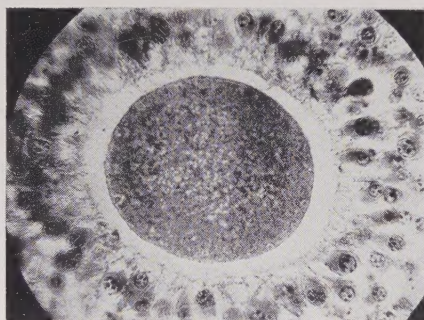
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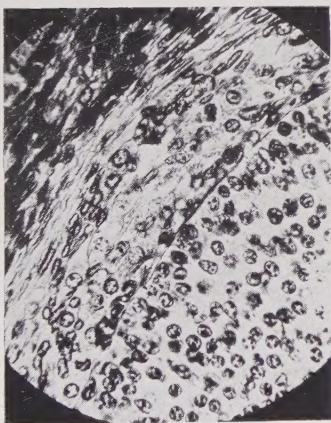
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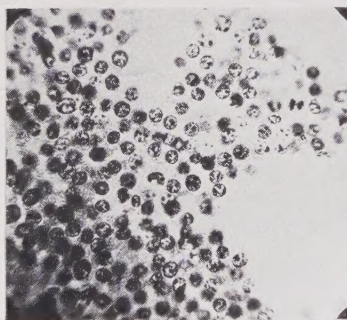
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